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Effects of 6-Benzylaminopurine and Salinity Stress on Flowering and Biochemical Characteristics of Winter Jasmine (*Jasminum nudiflorum* L.)

Masoume Fazeli[1], Davood Naderi[2*]

[1]Horticultural Department, Islamic Azad University,Isfahan (Khorasgan) Branch, Isfahan, Iran
[2]Young Researchers and Elite Clubs, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran

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*Corresponding author's email: d.naderi@khuisf.ac.ir

Abstract

The purpose of this study was to evaluate the effects of different levels of salinity stress and foliar application of 6-benzylaminopurine (benzyl adenine) on morphological and biochemical characteristics of winter jasmine in an experiment conducted in the Greenhouse Production Research Center of Islamic Azad University, Isfahan (Khorasgan) Branch in 2016-2017 growing season. The experiment was in a factorial form based on a completely randomized design with four levels of salinity stress (2, 4, 6 and 8 dS m^{-1}) as the first factor and three levels of benzyl adenine (0 as control, 25 and 50 mg l^{-1}) as the second factor in three replications (a total of 36 plots). The recorded traits included flower fresh and dry weight, flower number, the content of chlorophyll a, b, and total, carotenoids, and superoxide dismutase (SOD) enzyme of winter jasmine plant. The results showed that salinity levels and benzyl adenine foliar application had a significant effect on all traits. The activity of SOD was significantly higher in plants treated with 8 dS m^{-1} salinity than those treated with 2 dS m^{-1}, and the highest enzymatic activity was recorded at the salinity stress level of 8 dS m^{-1}. With respect to the effects of foliar application of benzyl adenine treatment, it was observed that this factor had positive concentration-depended effects on all traits. It can be concluded that although salinity stress had a negative effect on winter jasmine, benzyl adenine treatment minimized these adverse effects.

Keywords: BA, Chlorophyll content, Plant hormones, Superoxide dismutase, Tolerance of stress.

INTRODUCTION

Given the fact that plants need water, light, nutrient for maximum growth, development and yield, the excess or deficiency of any environmental factor may impair plant growth and development (Meena *et al*., 2017). On the other hand, plants are exposed to various factors and abiotic stresses (Rao *et al*., 2006) such as high or low temperature, salinity, drought, and other factors so that these factors create stressful conditions for plants (Crane *et al*., 2011; Jiang *et al*., 2016). In the meantime, salinity stress is one of the most important environmental factors and abiotic stress limiting germination, growth, and productivity of plants in the 21st century (Meena *et al*., 2017; Kumar *et al*., 2018; Shahmoradi and Naderi, 2018).

Some researchers have investigated the effects of different levels of salinity (control, 40 and 80 mM NaCl) on some traits of Kentucky bluegrass. The results have shown that higher concentrations of salinity stress were related to lower plant height, root and shoot dry weight, chlorophyll content, and leaf potassium content, whiles the application of the above treatments increased the electrolyte leakage, proline content, and sodium (Bayat *et al*., 2013; Arghavani *et al*., 2017). It has been found that salinity stress imposed its negative effects through ionic toxicity, osmotic stress, reduction of osmotic potential of soil solution (water stress), and secondary stresses such as nutrition imbalance, perturbation of the nutrition uptake, oxidative stress, or a combination of these factors (Ashraf, 1994; Pessarakli and Szabolcs, 2010). In addition, it has reported by Meena *et al*. (2017) that salinity stress happens due to unavailability of water and disruption of nutrient absorption and inflicts much damage to plant tissues and in the end, it affects productivity.

On the other hand, under these conditions, researchers are required to develop strategies to reduce the negative effects of salt stress (Acosta-Motos *et al*., 2017). It has been proven that in salinity stress, the accumulation of proline (Matysik *et al*., 2002), glycine-betaine and polyols in cells, the activation of antioxidant enzymes, and the synthesis of plant hormones are some well-known alleviation strategies for reducing the negative effects of salinity stress (Hoque *et al*., 2008). Furthermore, some researchers argue that the application of different levels of some plant hormones can help the plants in these conditions and these hormones can make a balance in the adverse effects of salt stress (Ryu and Cho, 2015; Khan *et al*., 2015). In addition, some researchers have shown that the foliar application of cytokinins in some plants could alleviate the negative effects of salinity stress (Ghorbani Javid *et al*., 2011).

Reports on the effects of cytokinin on salinity stress are contradictory. A study by Kirkham *et al*. (1972) on the foliar application of cytokinin to bean plants showed that plants treated with different concentrations of cytokinin were more susceptible to salinity stress than control plants, but wheat seedlings treated with cytokinin showed more tolerance to salinity stress than control (Nagavi *et al.,* 1982). Regarding the positive effects of cytokinins on reducing the negative effects of salinity stress, some researchers argue that cytokinins improve the growth and development of plants cultivated under salinity stress conditions (Sakhabutdinova *et al*., 2003). To support this finding, it has been reported that through enhancing root growth, leaf growth and photosystem II efficiency (Fv/Fm), cytokinins have a positive effect under salinity stress (Albacete *et al*., 2010; Ghanem *et al*., 2011) on vascular differentiation and nutrient mobilization (Fahad *et al*., 2015) and finally, they increase crop productivity under salinity conditions (Sampath Kumar *et al*., 2015).

According to the abovementioned cases about the effects of salinity stress on plants, it has reported that some ornamental plants are sensitive to salinity stress (Acosta-Motos *et al*., 2017). On the other hand, winter jasmine (*Jasminum nudiflorum* L.), which is an ornamental shrub from the olive family (Taghavi and Hasani, 2012), is relatively resistant to soil and water salinity (Vernieri *et al*., 2010). To support this claim, Ferrante *et al*. (2011) examined the effects of seawater salinity on the morphological and biochemical characteristics of six ornamental plant species (*Acacia cultriformis*, *Callistemon citrinus*, *Carissa edulis microphylla*, *Westringia fruticosa*, *Gaura*

lindheimeri, and *Jasminum sambac*) and observed that under the use of seawater salinity, the leaves of all studied plants became necrotic and lost their chlorophyll content and chlorophyll fluorescence, but the researchers recorded the lowest damage in *Jasminum sambac* plant among all studied species.

Despite numerous studies on the effect of plant hormones, such as cytokinin, on plants under different abiotic stresses, few studies have focused on ornamental plants so that there is no report about the effect of cytokinin on winter jasmine under salinity conditions. Hence, the present study was conducted to investigate the effects of different concentrations of benzyl adenine (BA) in the presence of different levels of salinity stress on morphological and physiological characteristics of winter jasmine and the response of winter jasmine to this treatment.

MATERIALS AND METHODS

Plant material, planting, and plant growth conditions

The winter jasmine plants in the present study were selected from two-year-old plantlets, which were purchased from "Erfan" Greenhouse located in Karaj. The plants were cultivated on December 10, 2017, and salinity stress and foliar application of BA were applied from the 20th of December 2017 (for 2 months). The experimental design was factorial in a completely randomized design with three replications conducted in Research Center of Greenhouse Production of Islamic Azad University, Isfahan (Khorasgan) Branch with a relative humidity of 40% and an average temperature of 28 ± 2ºC during the 2016-2017 growing season.

Plant materials

Three concentrations of BA (0, 25 and 50 mg L^{-1}) and four levels of salinity stress (2, 4, 6 and 8 ds m^{-1} NaCl) were selected. Before the experiment commenced, a sample of the soil and water used in the present study was tested. Based on the soil test, nutrients and fertilizers were applied to the soil in each of the pots. The results of the soil test are presented in Table 1 and the results of water analysis in Table 2.

Table 1. Physical and chemical characteristics of soil sample.

Soil texture	pH	O.M (%)	O.C (%)	EC(dS m^{-1})	CEC (meq $100g^{-1}$)	N (%)	P(mg kg^{-1})	K(mg kg^{-1})	Na(mg kg^{-1})	Fe (mg kg^{-1})	Cu(mg kg^{-1})	Zn (mg kg^{-1})	Mn(mg kg^{-1})
Loam-clay	7.67	4.62	2.69	2.96	24.7	0.27	21.35	700	1.32	2.45	5.73	0.668	1.3

O.M= Organic matter; O.C= Organic carbon;

Table 2. Chemical characteristics of water sample.

pH	EC (µmho cm^{-1})	Bicarbonates (meq l^{-1})	K (meq l^{-1})	Ca (meq l^{-1})	Mg (meq l^{-1})	Cl (meq l^{-1})	Na (meq l^{-1})
7.38	253.98	1.94	0.87	7.8	12.6	4.6	3.56

At the end of the experiment, some traits including stem height, flower fresh and dry weight, number of flowers, chlorophyll a and b, total chlorophyll, carotenoids, and SOD enzyme were measured by the following methods and formula.

MEASUREMENTS

Number of flowers

The number of flowers was counted on each plant per pot at the end of the experiment.

Fresh and dry weight of flowers

At the end of the experiment, to measure the fresh weight of flowers, flowers were selected from each pot and their fresh weight was measured using a Mettler Toledo scale with a precision of 0.001 g and the results were recorded. Then, in order to estimate flower dry weight, the flowers of each pot were placed in a special paper pocket and were dried in an oven (model UF_3OPlus, Memmert Company, Germany) at 70 °C for 48 hours.

Content of chlorophyll a, b and total chlorophyll and carotenoids

Chlorophyll a and b and total chlorophyll were calculated by the method of Arnon (1949) and carotenoids contents were obtained by Lichtenthaler (1987)'s method. Accordingly, 0.1 g of fresh tissue was first weighed and it rubbed in a stone mortar by the application of acetone 80%. Then, the extract was reached to 10 ml by adding acetone 80%. Immediately, some of the extract was transferred to a cell and absorbed by a spectrophotometer (model JENWAY 6300, UK) at 470, 645 and 663 nm. Again, acetone 80% was used as a blank solution. Finally, chlorophyll a and b, total chlorophyll, and carotenoids were determined in each sample by using the following formulas:

$$Chlorophyl\ a = \frac{[(12{,}7 \times D663) - (2{,}69 \times D645)] \times V}{1000 \times W}$$

$$Chlorophyl\ b = \frac{[(22{,}9 \times D645) - (4{,}93 \times D663)] \times V}{1000 \times W}$$

$$Total\ chlorophyl = \frac{[(20{,}2 \times D645) - (8{,}02 \times D663)] \times V}{1000 \times W}$$

$$carotenoids = \frac{[(1000 \times D470) - (1{,}82 \times Chl, a) - (85{,}02 \times Chl, b)]}{198}$$

V: Final volume of extracts per milliliters, W: Tissue weight per gram, D: Optical absorption.

Superoxide dismutase (SOD) enzyme activity

To measure SOD enzyme activity, a reaction mixture containing 50 mM of phosphate buffer, 0.013 mM of methionine, 0.1 µm of EDTA, and 2 µm of riboflavin was prepared and kept in full darkness. Immediately after adding the riboflavin, 3 ml of it was poured into a test tube and each sample was added with 100 ml of protein extract. The test tubes were placed at a distance of 30 cm from the light source for 16 minutes and at this time, the spectrophotometer was set at 560 nm with a dark solution as the control treatment. After 16 minutes, the samples were read at the mentioned wavelength. Finally, the SOD enzyme activity was calculated based on the enzyme unit per mg of protein for all samples (Giannopolitis and Ries, 1997).

Statistical analysis

The experimental design was conducted using a factorial based on a completely randomized design in three replications with two factors so that the first factor was salinity stress at four levels and foliar application of BA on winter jasmine was the second factor. The data were analyzed by SAS software (ver 9.4) and the LSD test was used at $P<0.05$ to compare the means of the data.

RESULTS

Number of flowers

Analysis of variance of flower number showed that although the salinity and BA treatments had a significant effect on flower number at $P<0.01$, the interaction of the salinity × BA was not significant for this trait (Table 3).

Table 3. Analysis of variance of winter jasmine morphological and biochemical traits under salinity stress levels, foliar application of BA at different rates and their interaction.

S.o.V	df	Flower number	Fresh weight of flower	Dry weight of flowers	Chloro-phyll α	Chloro-phyll b	Total chlorophyll	Carotenoids	SOD activity
Salinity (S)	3	49.44 **	1.55 **	0.48 **	146.19 **	61.91 **	384.22 **	13.50 **	196.03 **
BA (C)	2	34.03 **	3.78 **	0.90 **	184.38 **	15.82 **	295.97 **	8.07 **	373.05 **
S × BA (C ×S)	6	4.44 ns	0.38 **	0.04 ns	4.55 ns	0.31 ns	4.86 ns	0.31 ns	18.72 ns
Error	24	2.86	0.13	0.03	8.09	1.95	9.71	0.89	19.91
CV (%)		21.67	18.52	28.05	23.41	27.17	18.03	24.86	19.24

ns: Non-significant, * and **: significant at $P<0.05$ and $P<0.01$.

The results of means comparison showed that different levels of salinity had negative effects on flower number of winter jasmine plants. The maximum flower number was observed in plants treated with 2 dS m^{-1} but it did not have a significant difference with the number of flowers in 4 dS m^{-1} salinity (with 9.22 flowers). The minimum was obtained from the application of the 2 dS m^{-1} level (4.89 flowers) which had a significant difference compared with other treatments. On the other hand, according to the results (Table 4), the application of BA has a positive effect on flower number of winter jasmine per plant so that this index increased significantly by increasing BA concentrations. The highest flower number was observed in the application of 50 mg L^{-1} BA (9.33 flowers). Also, based on the present results, there was no significant difference between the number of flower in this treatment and that of the plants treated with 25 mg L^{-1} BA (8.08 flowers). Also, the minimum flower number (6 flowers) was obtained from the control treatment which had a significant difference with the number of flowers in other treatments (Table 4).

Fresh weight of flower

Based on the results in Table 3, there was a significant difference in flower fresh weight at $P<0.01$ under application of the different levels of salinity, different concentrations of BA, and their interaction (Table 3).

Means comparison of salinity levels on fresh weight of flower (Table 4) showed that salinity stress had negative effects on this trait so that the lowest fresh weight was obtained at 8 dS m^{-1} salinity treatment (1.02 g), but it did not differ from that of plants exposed to 6 dS m^{-1} (1.32 g) significantly. In addition to the above result, the highest fresh flower weight was recorded in the treatment of 2 dS m^{-1} salinity (2.01 g) so that a significant difference was observed in this treatment with other treatments. On the other hand, in investigating the effects of BA on fresh flower weight, the maximum of fresh weight (1.96 g) was achieved in the application of 50 mg L^{-1} BA and had a

Table 4. Means comparison for salinity stress levels, BA concentrations and their interaction on flower number and fresh and dry weight of flower traits in winter jasmine.

Treatments	Flower number	Fresh weight of flowers (g)	Dry weight of flowers (g)
Salinity (dS m^{-1})			
2	10.11 a	2.01 a	0.92 a
4	9.22 a	1.51 b	0.77 a
6	7.00 b	1.32 bc	0.49 b
8	4.89 c	1.02 c	0.42 b
LSD	1.65	0.35	0.18
Foliar application of BA (mg L^{-1})			
0	6.00 b	0.86 c	0.35 c
25	8.08 a	1.59 b	0.72 b
50	9.33 a	1.96 a	0.89 a
LSD	1.43	0.30	0.15

Mean of each variable fallowed by the non-similar letters have significantly difference at $p<0.05$

significant difference with other concentrations (fresh weight of flower equal to 0.86 g in control and 1.59 g in 25 mg L^{-1} BA) and the lowest fresh flower weight was achieved in control treatment that had a significant difference with the fresh flower weight of other treatments (Table 4).

Interaction between different levels of salinity and BA (Fig. 1) showed that the highest fresh flower weight (3.02 g) was achieved in 2 dS m^{-1} salinity × 50 mg l^{-1} of BA and had a significant difference compared the other interaction treatments. On the other hand, the lowest amount of this index (0.77 g) was obtained under 8 dS m^{-1} salinity × no BA application (Fig. 1).

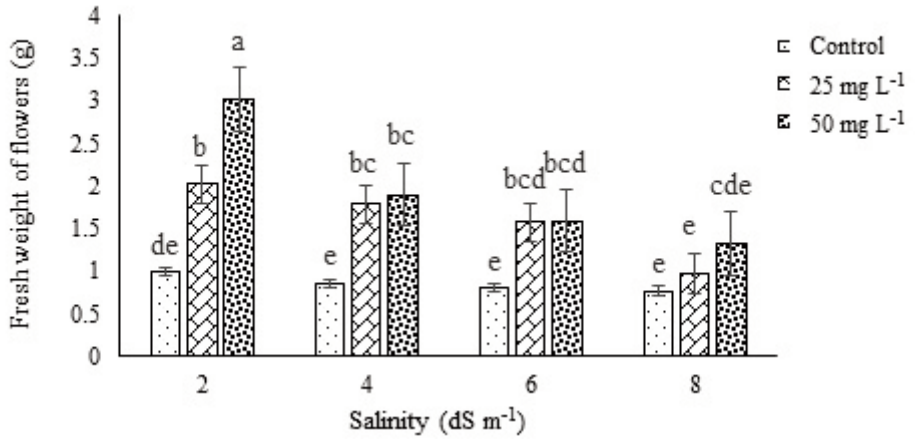

Fig 1. The effect of interaction of salinity × BA on fresh weight of flowers in winter jasmine. In each column, means with the similar letter(s) are not significantly different ($P<0.05$) using LSD test.

Dry weight of flower

According to the results, it can be concluded that salinity and BA had a significant effect on the dry weight of flowers at $P<0.01$, but interactions did not cause significant differences in this trait (Table 3).

Regarding the effects of studied treatments on dry weight of flower in winter jasmine (Table

4), means comparison for the use of salinity different levels showed that the highest flower dry weight was seen at 2 dS m^{-1} level, but it was not significantly different from the application of 4 dS m^{-1} salinity. On the other hand, the lowest dry weight of flower was achieved at 8 dS m^{-1} level but did not show a significant difference with 6 dS m^{-1} salinity. Apart from the above results, it can be observed that the highest dry weight of flower (0.89 g) was obtained at 50 mg l^{-1} of BA, showing a significant difference with other treatments, while the lowest (0.35 g) was for control with a significant difference with other treatments (Table 4).

Chlorophyll a, b and total chlorophyll

The results of ANOVA in Table 3 indicated that both different levels of salinity stress and different concentrations of BA caused significant difference at $P<0.01$ in the content of chlorophyll α, chlorophyll b, and total chlorophyll, but the interaction of salinity × BA was not significant for these three traits (Table 3).

Means comparison (Table 5) indicated that the highest amount of chlorophyll *α* (16.49 mg g^{-1} F.W.) was achieved at salinity level of 2 dS m^{-1} which had a significant difference with other treatments. The minimum content of chlorophyll α was recorded under 8 dS m^{-1} (6.77 mg g^{-1} F.W.) salinity, showing significant differences with other treatments. In investigating the effect of BA concentrations on chlorophyll α, based on the results in Table 5, the highest chlorophyll α was obtained at 50 mg l^{-1} concentration (16.63 mg g^{-1} F.W.). Also, the results indicated a significant difference in chlorophyll *α* in 50 mg L^{-1} treatment compared to that of the other BA concentrations. In addition, in Table 5 we see that the lowest amount of chlorophyll α (9.37 mg g^{-1} F.W.) was obtained under no application of BA (control treatment), but no significant difference was observed between this value and the amount of chlorophyll α under the application of BA in 25 mg l^{-1} concentration (10.44 mg g^{-1} F.W.).

Table 5. Means comparison for salinity stress levels, BA concentrations, and their interaction on chlorophyll α, chlorophyll b, total chlorophyll, carotenoids and SOD activity in winter jasmine.

Treatments	Chlorophyll α (mg g^{-1} F.W.)	Chlorophyll b (mg g^{-1} F.W.)	Total chlorophyll (mg g^{-1} F.W.)	Carotenoids (mg g^{-1} F.W.)	SOD (mg g^{-1} pro F.W.)
Salinity (dS m^{-1})					
2	16.49 a	8.62 a	25.11 a	5.34 a	18.67 b
4	13.09 b	5.52 b	18.61 b	4.12 b	20.29 b
6	12.25 b	3.88 c	16.13 b	3.9 bc	24.76 a
8	6.77 c	2.54 c	9.31 c	2.47 c	29.03 a
LSD	2.77	1.36	3.03	0.92	4.35
BA (mg L^{-1})					
0	9.37 b	3.93 b	13.30 b	3.19 b	16.99 b
25	10.44 b	5.26 b	15.70 b	3.48 b	24.75 a
50	16.63 a	6.22 a	22.85 a	4.74 a	27.81 a
LSD	2.39	1.18	2.63	0.80	3.77

*In each column, means with the similar letter(s) are not significantly different ($P<0.05$) using LSD test.

With respect to the effects of salinity stress on chlorophyll b (Table 5), the results showed that the highest and the lowest content of chlorophyll b (8.62 and 2.54 mg g^{-1} F.W.) were obtained from 2 and 8 dS m^{-1} salinity, respectively. Also, according to Table 5, we can see that there was a significant difference between the content of chlorophyll b under 2 dS m^{-1} treatment and other

treatment but the results on the content of chlorophyll b under 8 dS m^{-1} salinity revealed no significant difference between this value and chlorophyll b under 6 dS m^{-1} salinity (2.54 mg g^{-1} F.W.). Moreover, means comparison for the effect of different levels of BA on chlorophyll b (Table 5) showed that with the increase in BA concentrations, the amount of chlorophyll *b* significantly increases so that its highest content (6.22 mg g^{-1} F.W.) which had a significant difference with the other treatments was obtained under the application of 50 mg l^{-1} BA and the lowest chlorophyll b (3.93 mg g^{-1} F.W.) was achieved in control treatment (no application of BA). According to the above results, it can be observed that there was not a significant difference between the content of chlorophyll *b* at control treatment and 25 mg l^{-1} (5.26 mg g^{-1} F.W.) concentration (Table 5).

Means comparison for the effects of salinity stress on total chlorophyll in winter jasmine leaves (Table 5) indicated a significant decrease in this trait under different levels of salinity stress so that higher salinity levels were related to significantly lower total chlorophyll content. Based on the results, the maximum content of total chlorophyll (25.11 mg g^{-1} F.W.) was observed at 2 dS m^{-1} level which had a significant difference with the other treatments and the minimum content of total chlorophyll (9.31 mg g^{-1} F.W.) that had a significant difference with other treatments was obtained from 8 dS m^{-1} salinity level. On the other hand, in studying the effects of BA concentrations on total chlorophyll, it can be seen that the highest total chlorophyll was recorded at 50 mg l^{-1} concentration (22.85 mg g^{-1} F.W.) which had a significant difference with other treatments of BA concentrations and the lowest content of total chlorophyll was obtained from control treatment (13.30 mg g^{-1} F.W.), but it is observed that there is no significant difference in the content of total chlorophyll between this treatment and the application of 25 mg l^{-1} (15.70 mg g^{-1} F.W.) (Table 5).

Content of carotenoids

The results of ANOVA in Table 3 indicate that different levels of salinity and BA concentrations caused significant differences in the content of carotenoids at $P<0.01$, but no significant difference was obtained under the application of salinity × BA interaction (Table 3).

With respect to the effect of salinity stress and foliar application of BA on carotenoids, it was seen that salinity stress had an adverse effect on carotenoids, but BA foliar application had a positive effect. Among different rates of salinity stress (Table 5), the highest content of carotenoids was obtained from 2 dS m^{-1} concentration (5.34 mg g^{-1} F.W.) and there was a significant difference between amount of carotenoids at 2 dS m^{-1} treatment with other levels of salinity stress. Also, the lowest amount of carotenoids (2.47 mg g^{-1} F.W.) was recorded at salinity level of 8 dS m^{-1}. On the other hand, we observed that with the increase in BA concentrations, the content of carotenoids increased significantly so that the highest amount of carotenoids (4.74 mg g^{-1} F.W.) which had a significant difference with that under other treatments of BA concentrations, was obtained from 50 mg l^{-1} BA and the lowest amount (3.19 mg g^{-1} F.W.), which had no a significant difference with that of the application of 25 mg l^{-1} BA (3.48 mg g^{-1} F.W.), was obtained from control treatment (Table 5).

SOD enzyme activity

According to Table 3, it was observed that although SOD activity was influenced by different levels of salinity and BA application significantly at $P<0.01$, their interaction was not significant for this trait (Table 3).

Means comparison (Table 5) about SOD activity shows a significant increase under the treatment of both salinity and foliar application of BA. We concluded that as salinity level was increased, the activity of SOD enzyme was increased so that the highest activity of this enzyme was observed at salinity level of 8 dS m^{-1} (29.03 mg g^{-1} pro F.W.). However, it did not differ significantly from the application of 6 dS m^{-1} salinity (24.76 mg g^{-1} pro F.W.). The lowest activity of SOD en-

zyme (18.67 mg g^{-1} pro F.W.) was observed at 2 dS m^{-1} salinity without any significant differences with the treatment of 4 dS m^{-1} salinity (20.29 mg g^{-1} pro F.W.). Also, as is evident in Table 5, it was observed that the highest activity of SOD enzyme under foliar application of BA was obtained at BA rate of 50 mg l^{-1} (27.81 mg g^{-1} pro F.W.) but it exhibited an insignificant difference with that of 25 mg l^{-1} BA (24.75 mg g^{-1} pro F.W.). As well, Table 5 reveals that the lowest activity of SOD enzyme (16.99 mg g^{-1} pro F.W.), which had a significant difference with the other concentrations of BA, was obtained from the control treatment (no application of BA).

DISCUSSION

Some researchers studying various plants have pointed out that salinity stress affects many aspects of plant growth and metabolism (Sivritepe *et al*., 2003; Vahdati *et al*., 2012; Bayat *et al*., 2013). Also, to survive under salinity conditions, plants react differently and change their morphological and physiological characteristics (Amirjani, 2010). In general, the common symptoms of salinity damage are growth retardation (initial damage leading to other complications) and the aging and death of plants exposed to salt stress in the long time (Jouyban, 2012). With respect to the effects of salinity stress on morphological indices and according to the results of the study (Table 4), salinity stress affected flower number and fresh and dry weight of winter jasmine so that the stronger the salinity stress was, the lower these traits were. The negative effects of salt stress have been confirmed by other researchers so that in this regard, some researchers have reported the reduction of plant growth in response to the factors described above such as ionic toxicity, mineral deficiency, physiological disorders, biochemical disorders, or a combination of these factors (Rajaravindran and Natarajan, 2012; Saleh, 2013). Also, Parida and Das (2005) point out that salinity stress limits plant growth and development and consequently reduces the fresh and dry weight of leaves, stems, and roots. On the other hand, it has been proven that there are different strategies to alleviate the impact of salinity stress on plants, one of which being the application of plant growth regulators (Miransaria and Smith, 2014; Geetha and Murugan, 2017). In the present study, with respect to the morphological traits, we observed that the application of BA reduced the adverse effects of salinity stress (Table 4). Regarding the effects of BA under salinity stress, it has been reported that cytokinins affects the growth and photosynthetic efficiency of leaves through vascular bundle of leaves and thereby increases the growth, development and yield of plants under salinity conditions (Albacete *et al*., 2010).

In general, it has been reported that chlorophyll is the main source of green color in photosynthetic systems of plants, and in undesirable environmental conditions, chlorophyll content is considered as an indicator of tolerance (Srivastava *et al*., 1988). Accordingly, Xu *et al*. (2000) reported that chlorophyll levels of leaves were reduced under salinity stress due to the decomposition of chlorophyll enzymes. Also, chlorophyll degradation due to the salinity stress has been reported in some varieties of salinity-sensitive crops (Hernandez *et al*., 1995). Sayyed *et al*. (2014) note that the loss of chlorophyll in plants is a sign of oxidative stress in plants. Therefore, it can be observed that the reduction of plant pigments such as chlorophyll and carotenoids (Table 5) under the use of salinity stress is consistent with some researches. Also, in Table 5, we observed that the application of BA alleviated chlorophyll destruction. In agreement with the effects of cytokinins in increasing the pigments, after extensive work, researchers have found that the common action of the plant hormones auxin and cytokinin in plants treated with environmental conditions is linked to plant growth responses to environmental changes (Kazan, 2013; Bielach *et al*., 2017).

Since chlorophyll is considered one of the parameters of salinity tolerance in plants (Srivastava *et al*., 1988), chlorophyll disintegration due to salinity stress has been reported in some saline-sensitive plants (Hernandez *et al*., 1995). It has been proven that salicylic acid (SA) exerts its influence on photosynthesis through its effect on photosynthetic rate, stomatal factors (Khan *et*

al., 2010), pigments, and the structure of chloroplasts and enzymes involved in the photosynthesis process (Rajasekaran *et al*., 2002; Poor *et al*., 2011). Also, the role of SA in defense mechanisms has been suggested under salinity stress (Tissa *et al*., 2000; Al-Hakimi and Hamada, 2001). With respect to the effects of cytokinin on reducing the negative impacts of salinity stress, it has been stated that cytokinin improves the resistance of plants to salt stress (Mok and Mok, 2001; Ghorbani Javid *et al*., 2011). It has also been proven that cytokinins can affect photosynthetic parameters, such as chlorophyll content, synthesis and degradation of photosynthetic proteins, electron transfer, and enzymatic activity, and thereby increasing the chlorophyll content under different concentrations (Pospisilova *et al*., 2005). In another study, it has been suggested that cytokinin reduces reactive oxygen species (ROS) in plants (Chakrabarti and Mukherji, 2003), and since salt osmotic stress induces oxidative stresses caused by ROS (Shahmoradi and Naderi, 2018), tolerance to salinity stress in plants exposed to cytokinin has been connected to the alleviation of the negative effects of above activity (Chakrabarti and Mukherjee, 2003).

Given that ROS increases chlorophyll degradation and reduces chlorophyll content of leaves (Sairam *et al*., 2002), the reduction of chlorophyll content in the present study (Table 4) seems to be natural. In the following plant responses, antioxidant enzymes such as catalase and superoxide dismutase can eliminate and deactivate ROS (Bailly, 2004). It has been documented that salinity stresses convert the content of ROS to hydrogen peroxide within the cell, which inhibits the activity of the Kelvin cycle. Ultimately, this action limited the process of sugar production in plants. Therefore, it increased the activity of antioxidant enzymes and prevented from harmful effects of hydrogen peroxide formation on the process of sugar production in chloroplasts (Shen *et al*., 1997). It is notable that the increase in the content of antioxidant enzymes in plants exposed to salinity stress is not the only mechanism of salinity tolerance, but this mechanism can be used in conjunction with other substances to enhance salinity-resistance of crops (Abo-Kassem, 2007). It has also been shown that superoxide dismutase is the first enzyme that converts into hydrogen peroxide, and H_2O_2 is converted to water and oxygen by the action of catalase or ascorbate peroxidase enzymes (Foyer *et al*., 1997). In addition to the above results, our results indicate that salt stress and the application of BA can increase the activity of SOD enzyme (Table 5). In this respect, as mentioned, plants have created different biochemical and molecular strategies to resist salinity conditions, and this action can be the synthesis of osmolytes, production of antioxidant enzymes, and the production of plant hormones (Ashraf and Mc-Nielly, 2004).

CONCLUSION

It can be concluded that one or all of the above factors had significant effects on morphological and biochemical traits in this study. In addition, although salinity levels had negative effects on morphological and biochemical traits in this study, the application of BA had a positive effect on alleviating salinity effects. Therefore, it can be advised to others that the use of different levels of BA reduces the effects of salinity stress and improves morphological and biochemical traits in winter jasmine plant.

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